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THREE NEW SPECIES OF AMEBAS: AMOEBA BIGEMMA NOV. SPEC., PELOMYXA LENTISSIMA NOV. SPEC. AND P. SCHIEDTI NOV. SPEC.

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The classification of the amebas[1] is peculiar in that two methods of species determination are followed. The larger amebas are classified according to the characteristics possessed by the cytoplasm and the general character of the vegetative stage, while the smaller amebas are being described according to the method of nuclear division prevailing during reproduction. In the larger amebas a study of nuclear division is extremely difficult and for many species impossible, owing for one thing to our ignorance of culture methods by means of which these species could be raised in abundance. On the other hand the small amebas exhibit so little cytoplasmic differentiation that specific determinations on this basis seem impossible.

A specific determination is interesting, however, from at least two points of view. One is the viewpoint of establishing blood relationships of descent between the different species, or systematics proper. The other point of view is a purely practical one, i.e., quick identification. The physiologist or the experimentalist wants a quick and correct method for identifying the organism he is working with. It is obvious therefore that if an ameba possesses characteristic cytoplasmic differentiations which may be observed at any time, the ameba will come to be recognized by these characteristics rather than by a complicated series of nuclear events which occur only occasionally and are frequently made out only with difficulty. In short, as a means of identification, cytoplasmic char-

[1] The word ameba is used as a common name for naked rhizopods lacking internal skeletons such as *Amoeba, Pelomyxa, Protamoeba, Endamoeba, Nägleria, Hyalodiscus, Dinamoeba, Vahlkampfia*, etc.

acters are preferable where they exist; where these do not exist, recourse may be had to the nuclear division process.

All the larger amebas possess cytoplasmic differentiations in sufficient number and conspicuousness to serve as a ready means of recognition. Many of these characters are subject to very slight variation as a result of changes in the environment. Individual isolation pedigrees carried on for upwards of a hundred linear generations together with many collateral lines under varying food conditions, showed that most of the cytoplasmic characters are hereditary and practically uninfluenced by what might be said to be the most common environmental changes (Schaeffer, Science, 1916, p. 468). Amebas are therefore in this respect like all other groups of animals and the method of classifying them according to cytoplasmic differentiations is therefore sound.

These considerations should convince especially our younger microscopists that the investigation of our larger amebas is not nearly as difficult or forbidding a field as might be imagined from the great amount of labor that has been expended on the study of the life histories and nuclear phenomena of some soil and parasitic amebas during the past decade. The fifty or so species of aquatic amebas thus far described represent beyond any question only a very small fraction of the number of species in existence, and this number of known species could probably be doubled within a few years by careful examination of our marshes and ponds.

AMOEBA BIGEMMA NOV. SPEC.

Diagnosis. Size in locomotion, 100 to 300 microns long. Form very changeable. Pseudopods, numerous, tapering, blunt, never with sharp points. Surface smooth, no fine folds or ridges. Endoplasm usually containing numerous small twin crystals; crystals attached to 'excretion spheres.' Movement rapid, about 125 microns per minute. Nucleus single, spherical or slightly ovoid, about 12 microns in diameter; chromatin in small masses clumped loosely together in the center of the nucleus in a nearly spherical mass about 6.5 microns in diameter. Contractile vacuoles small about 15 microns in diameter; numerous; no coalescence among them; systole slow. Endoplasm filled with small vacuoles. Food: flagellates, ciliates, diatoms, rhizopods, nematodes, vegetal tissue, etc.

This ameba, for which the specific name *bigemma* is proposed, resembles to some extent the figures and descriptions of Parona's *digitata*, Mereschkowsky's *angulata*, Gruber's *spumosa* and Penard's *vespertilio*. In fact I regarded it at first as the *angulata* of Mereschkowsky or the *vespertilio* of Penard, which it occurred to me might possibly be synony-

mous. But after further study of the characters of this ameba I began to suspect that my earlier conclusions regarding its specific reference might be mistaken. I accordingly investigated the specific characters of this ameba in connection with some experimental work, under widely varying conditions for about three years, and compared my observations with the published reports of earlier investigators of amebas with the result that I am unable to confirm the specific descriptions by any of the authors named from this ameba.

In the first place, Mereschkowsky's ('79) description is extremely vague (pp. 203-204) Mereschkowsky says the plasm of angulata is transparent, that it contains two kinds of grains: a few large ones and numerous small ones. About three contractile vacuoles and a small round nucleus are present. Few, "am ende zugespitzten (doch nicht wie bei A. *filifera* mit welcher *A. angulata* viel Aehnlichkeit hat) und die gestalt dicker, breiter Kegel habenden, vom Körper ausgehenden Pseudopodien characteristisch. diam. 0.0235." Movements very rapid. The figure illustrating this description is very crude. With the exception of size, this description as far as it goes might apply to a number of species of amebas. The size, 23.5 microns, is very much smaller than that of *bigemma.*

Parona's description of *A. digitata* (1883. Essai d'une Protistologie de la Sardaigne. Arch. des Science physiques et naturelles. T. 10. Troisième periode. p. 225-243. 1 plate) is somewhat more definite than Mereschkowsky's of *angulata. A. digitata* possesses a very granular endoplasm, a rounded and conspicuous nucleus, a large contractile vacuole, "pseudopods longs, conique et aigus," pseudopods always in small number. Movement is rather slow. Size, 63 microns (p. 228). The only three characters which may be considered distinctive are the size, the conical and pointed (the figure shows needle points) pseudopods, and the number of contractile vacuoles. None of these characters however are found in *bigemma.* Parona makes no mention of vacuoles in the endoplasm, which, if he had seen a *bigemma,* he could not have helped seeing, since these vacuoles are quite as conspicuous as the nucleus. There can be little question, I think, that Parona described another ameba than *bigemma* under the name *digitata.*

Leidy(79) figures several amebas resembling *bigemma, vespertilio* and *digitata* more or less closely, but he regarded them all as varieties of *proteus,* or as forms of uncertain specific reference.

In his description of *A. spumosa* Gruber is no more explicit than Mereschkowsky in the instance mentioned. *Spumosa* has broad flat pseudopods, a vesicular nucleus, an endoplasm filled with vacuoles, no granules, a size of 25 microns, according to Gruber. As emended by Penard ('02) *spumosa* possesses these characteristics: A length of from fifty to one hundred and twenty-five microns; form resembling the foot of a goose, with very fine longitudinal lines on the surface; numerous vacuoles; contractile vacuole as much as thirty microns in diameter; a great many bicuspid granules of very small size in the endoplasm; nucleus deformable like that of *A. limax*; a compact nucleolus with a narrow margin of nuclear sap between it and the nuclear membrane. Althought I am inclined to accept the emendation of Penard because of its making for greater definiteness and stability in this difficult genus, yet it appears to me that instead of really emending or elaborating Gruber's description, he actually describes a new and different species. It is evident that another ameba than *bigemma* was under observation by both Gruber and Penard when these authors wrote their descriptions of *A. spumosa.*

Penard's description of *A. verspertilio* (1902, Faune Rhizopodique du bassin du Leman. Geneva, pp. 714) is as follows: size, about seventy microns length; pseudopods have always a conical form, their extremities being usually sharp pointed although the point may be slightly rounded occasionally for a moment[2]; posterior end sticky, dragging debris along as it moves forward; a profusion of extremely small green grains, and sometimes large excretion grains, in the endoplasm; a sperical nucleus with a compact nucleolus which is often covered with fine points; contractile vesicles one, two or three; almost always a considerable number of vacuoles appearing and disappearing as if they were contractile in the endoplasm (pp. 92-95). Penard's figures (p. 94) resemble in a general way the figures of *bigemma*, but in the important points such as the number and character of vacuoles, the shape of the pseudopod extremities, the relative diameters of the nuclear membrane and the nucleus, the character of the "nucleolus," stickiness of the posterior end, inclusions, etc., there is little resemblance between *vespertilio* and *bigemma.*

[2] . . . les pseudopodes ont toujours une forme conique, anguleuse; leur extermite est en principe acérée; mais parfois la pointe peut s'arrondir pour un instant (p. 93). His figures all show sharp needle points on the extremities of the pseudopods.

The *Amoeba bigemma* is of medium size, being usually from 100 to 300 microns long in locomotion. Occasionally the size is very much greater. In several old cultures the amebas frequently arrived at a length of 500 microns in locomotion. As a rule, the average size in new cultures is about 150 microns.

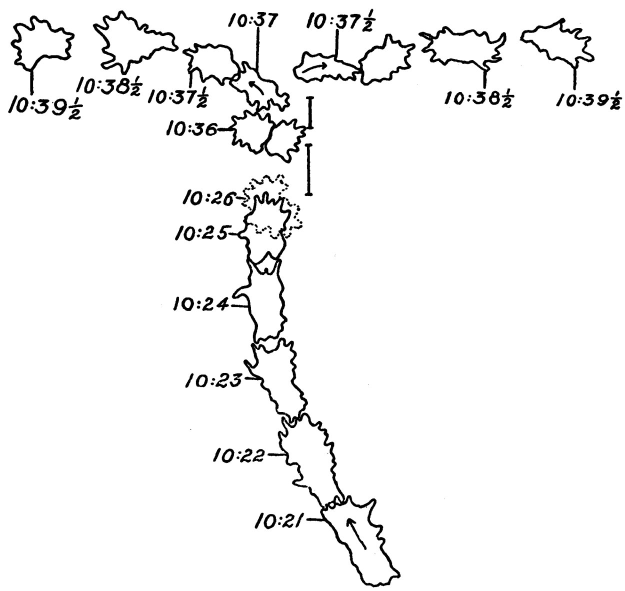

Fig. 1. Camera lucida drawing of an *A. bigemma* in locomotion during the process of fission. The figures indicate the time in hours and minutes. The drawings from 10:37½ on were moved up from their true position the length of the vertical line below; likewise for all drawings from 10:36 on. Note slowing down in the rate of locomotion as the fission crisis was approached, and the gradual increase of speed after division.

Although the general shape of this ameba is subject to very great variation, yet the various transformations are very characteristic. Perhaps the most characteristic feature of these transformations is the tongue like pseudopods, usually short, sometimes very long, which are continually being thrown out at the anterior end and on the free surface during locomotion (Pl. VII, fig. 2). These projections are frequently more or less conical, though more often perhaps they have somewhat the shape

of a hart's tongue in outline. These projections are not pseudopods in the same sense that the projections in *dubia* or in *proteus* are pseudopods. In the latter species a pseudopod gives direction to locomotion, that is, the whole ameba frequently flows into a pseudopod. In *bigemma*, however, the whole ameba almost never, if at all, flows into a pseudopod; but the tips of the pseudopods advance at about the same rate of speed as the anterior end of the ameba as a whole advances. The pseudopods are, therefore, to be regarded rather as "toes" on the single pseudopod of which this ameba usually consists. During active locomotion the general shape of the body is most frequently triangular, with the broad base advancing, and more or less compressed. When the animal is disturbed it assumes a spherical shape. When suspended in the water for some time the ameba assumes a shape closely resembling that of the rayed state of a *radiosa*. From a central spherical mass of about thirty or forty microns diameter, long, slender, tapering pseudopods are thrown out which are of a much more permanent character than the pseudopods thrown out during locomotion. These projections are sometimes perfectly straight and of equal size and disposed opposite to each other on the spherical mass. More often however they are curved and of irregular shape and size. Their number is usually from six to eight. All the pseudopods formed during locomotion or while suspended are blunt, very definitely blunt. Of all the thousands of individuals which I have examined in all the different stages and cultural conditions, I have never seen any but definitely obtuse pseudopods. The photographs of live amebas, figs. 4 and 5 indicate the degree of obtuseness characteristic of the pseudopods of this species (Pl. VII).

The streaming of protoplasm during locomotion presents some points of interest. One does not observe the endoplasm flowing slowly in a definite direction in this species as one may in *proteus* or *dubia*, for example, but the streaming is jerky and irregular. The endoplasm seems to drain from the sides and posterior end toward the anterior end against numerous obstructions which often give way. Thus there are developed momentary counter or cross currents and eddies. The peculiar character of the streaming is due to several causes. In the first place the numerous pseudopods are formed and retracted without definite reference to the direction of locomotion. Then again the upper surface of the ameba is not level but extremely irregular; one observes a confusion of high ridges and deep depressions thrown together without

observable order. From the depressions more or less permanent pillars of stiffened ectoplasm pass down to the lower surface obstructing the flow of endoplasm. When these pillars give way, as they frequently do, the bottom of the depression is suddenly pushed upward as if the endoplasm were under considerable internal pressure. Another cause of the irregularity of endoplasmic streaming is due to the fact that the anterior end does not advance steadily over the whole front, but by a series of waves here and there. Such a wave consists usually of a web of ectoplasm flowing out between and connecting two or more of the pseudopods; then the web halts momentarily while the pseudopods push out again or while new pseudopods form.

The rear end of the ameba usually plays little part in locomotion in a special way; usually the rear end is smooth and free from any pseudopodial projections. Occasionally, however, a long thin flat pseudopod may be rapidly thrown out near the posterior end which fastens itself to the substratum so well that considerable force is required to dislodge it (fig. 2). The ameba sometimes becomes stretched out to several times its usual length before the pseudopod is pulled loose. This behavior also results often in another phase of movement that I have much more frequently observed in this ameba than in any other, viz., some of the endoplasm begins to flow toward the posterior end until the ameba is cut almost in two in the middle. The connection is so thin that one looks every second for the connecting strand to break, but it does not break. Sooner or later one or the other portion of the endoplasm flows back and the whole mass again becomes unified in streaming.

The rate of movement is rapid, being about 125 microns a minute. Although the anterior end advances very unevenly and irregularly, there are, nevertheless, long segments in the path of an ameba moving on a plane surface that are straight. There is present in this ameba, therefore, the same tendency to keep on moving in the direction in which it started to move as there is in *proteus* (Schaeffer, '12, '18; this point will also be discussed at length in a paper soon to be published).

There is present in this ameba a layer of very thin protoplasm on the outside of the ectoplasm, as usually defined, which moves forward over the ameba in the same direction as that in which the ameba moves, but at a more rapid rate. This is clearly shown by the forward movement over the ameba of small particles which cling to this layer of protoplasm (Schaeffer, '17).

During locomotion there is usually a broad zone of clear ectoplasm at the anterior end. Not only are the smaller pseudopods in this region free from granules but the intervening spaces also consist of ectoplasm.

The nucleus is easily seen. Unless obscured by food masses photomicrographs of the living amebas usually show it (fig. 4.). It is spherical or very slightly ovoid (fig. 3). In an ameba of about 200 microns length the nucleus is about twelve microns in diameter. The chromatin consists of very small ovoid granules collected together in the centre of the nucleus in a slightly irregular oval mass of about six or seven microns diameter. The color of the chromatin is a pale bluish yellow green. Between the chromatin mass and the nuclear membrane, which is perfectly transparent, there is a zone of clear nuclear sap. The nucleus is single, though occasionally an ameba is found with two nuclei, which statement is true of course for practically all amebas. The nucleus is deformable though it is not often that one observes striking deformations as it is swept along by the endoplasm.

In one culture of large amebas of this species the nuclei were about twenty-eight microns in diameter, and the chromatin mass of irregularly spherical shape was about fourteen microns in diameter. When these amebas were slightly squeezed under the cover glass one or two masses of mostly perfectly homogeneous pale bluish yellow green material was pressed out from the chromatin mass. There were present here and there large spheres of denser material of the same color as the homogeneous masses. Both the granular mass and the homogeneous masses rounded themselves up and collected near the centre of the nucleus. The material making up the masses seemed to be of the same sort, though I did not employ staining methods to determine whether the interior of the granular chromatin mass was really chromatin or some other substance. It is, however, interesting to know that at least some of the material inside the chromatin mass is not granular while the outside material is.

There seem to be three kinds of vacuoles in this ameba in so far as their functions are concerned. The endoplasm contains scattered about in it everywhere numerous (100 or more) small clear vacuoles of various sizes, mostly under ten microns in diameter, which may be called *permanent* vacuoles. What the function of these vacuoles is, or what conditions are necessary to their origin, remains unknown. That all of these may become contractile is hardly possible, unless they retain their identity in the ameba's body for many hours.

The second kind of vacuoles are the *contractile* vacuoles. These have the same general appearance as the permanent vacuoles, excepting that they are more refractive to light. These vacuoles arrive at a diameter of about fifteen microns before they contract. It is very seldom that a larger size is attained. The diastole of these vacuoles is rather slow. The systole is also very slow, occupying from two to six seconds. There are a number of contractile vacuoles present at one time. Under favorable conditions as many as four may be observed to be in the process of diastole at one time. The general appearance of the later stages of a contractile vacuole, that is, higher refractive index of its contents, possibly indicates that these vacuoles are different from the permanent vacuoles from their beginning.

The third kind of vacuole, which may be called the *fecal* vacuoles, are not frequently met with in amebas. These are large spherical vacuoles containing in proportion to their size a very small amount of fecal matter. These may reach a diameter of twenty or twenty-five microns and in occasional large specimens a diameter of from forty to sixty microns. I have not been able to ascertain whether the vacuole originates around the fecal matter or whether after the vacuole is partly formed the fecal matter is voided into it. Since however, the vacuole usually becomes very large, it is evident that the later stages of the increase in size is due to the contained solid matter. The systole of these vacuoles is very slow. The liquid contents is first expelled and then after a pause the solid matter is thrown out in the way common to amebas generally.

None of these different kinds of vacuoles seem to grow by coalescence; at least from extended observation with this point in view I have never seen a single case of coalescence, although vacuoles frequently remain in close contact for a minute or longer.

Another very interesting element in the endoplasm is the crystals. These are usually very numerous and conspicuous, ranging in size from one and one-half to two and one-half microns in length. The general shape is like that of an hour glass or dumb-bell. They seem to be formed of two bicuspid crystals attached to each other by their apices (figs. 2, 3). Under ordinary light they appear dark gray in color; but in polarized light they show up very brightly, and then their twin structure becomes very evident. This is the only ameba known in which such a twin structure of crystal formation is found. The polariscope shows an

unmistakable twin structure, however, in only about half the cultures I have so far investigated. In the others the two points of light are joined by a bar of light so that one sees a band of light not constricted in the middle. It may be inferred, however, that in these cultures the earlier stages of crystal formation are also on the twin pattern. It has been found that the character as well as the amount of food influences the size and to some extent the character of the crystals formed. Perhaps the most striking thing about the crystals in this ameba is the fact that they are always attached to the so-called excretion spheres when these are present, as they nearly always are (fig. 3). There is never but one crystal attached to a sphere. The size of the sphere bears no relation to the size of the crystal, the spheres being in some cases just barely visible, while the crystals may be two microns long. When the the spheres are small the crystals are always attached to them at their middle. The sphere and crystal bear a remarkable resemblance to a fish embryo lying on its egg yolk, and they form interesting objects for observation as they tumble along in the streaming endoplasm. Occasional twin crystals are observed apparently free from attachment to spheres, but it is possible that the spheres are extremely minute in such cases, too small to be seen. The excretion spheres rarely exceed a diameter of three microns. In some cultures a few bicuspid crystals with irregular sides are observed occurring singly. The maximum size of these is about two and one-half microns. Occasionally also two twin crystals are found crossed, (fig. 3). Altogether, the crystals form the most definite specific character of this ameba, and the presence of such crystals attached to spheres in an ameba may be regarded as definitely proving its specific identity.

This species is a voracious feeder. Flagellates, shelled rhizopods ciliates, rotifers, nematodes, diatoms, etc., and especially bits of vegetal tissues and masses of bacteria, are readily devoured. The body is frequently stuffed with food.

This ameba is one of the hardiest known to me. I have kept numerous and continuous cultures, after being well established, for several years without much difficulty. The species is subject to very little variation excepting size. In nature this species must be considered rare, though it is found frequently where large masses of vegetation are undergoing decay in quiet water.

Pelomyxa lentissima nov. spec.

Diagnosis. Length in locomotion, 75 to 125 microns. Body usually very much compressed and applied closely to substratum. Changeable in shape, general outline oval with few pseudopods. Quiescent stage with numerous pseudopods of various shapes. Color of body brownish; of protoplasm, pale bluish. Uroid of fine or large root like projections. Rate of movement very slow, from 1 to 2 microns per minute. Nucleus spherical, about 14 microns in diameter. Chromatin in a spherical layer of granules about 11 microns in diameter, with spherical body about 2.5 microns in the centre of the nucleus. Two nuclei frequently present. Contractile vacuoles numerous, 40 or more; maximum size about 10 microns; systole sudden; diastole very slow. Numerous or few small irregular crystalline masses present. Numerous bacterial rods of about 4 microns length present. Only very few refractive (starch) bodies present.

This pelomyxa is readily recognized by its small size and its very slow rate of locomotion (Pl. VIII, fig. 6). It is, in fact, much the slowest moving pelomyxa thus far reported, and I, therefore, propose the specific name of *lentissima* for this species.

I have found this organism in large numbers in old cultures from the marshes of Lonsdale on several occasions. But on account of its small size and its habit of flattening itself out on and sticking close to the surface on which it moves, it more readily escapes detection than other amebas of the same size. The color of this species is a dull brownish, somewhat like that of *P. belevskii,* but not so deep a shade, owing to its smaller size. This color is, of course, due to the contained food materials and the indigestible remains of food objects. It seems to be a habit of this and other pelomyxas to carry for a long time indigestible materials from food objects before excreting them, if indeed some of this material is ever excreted while the animal is in the vegetative stage. The color of the protoplasm is of a bluish violet tint.

This pelomyxa as distinguished from the other species, flattens itself out and sticks very close to the surface during locomotion. At such a time it is thickest in the centre and gradually becomes thinner as the periphery is approached. Around the entire animal there is a clear zone of protoplasm which is hyaline and very thin and of which the exact outer limit is very difficult to see. Pseudopods are continually being extended and retracted from the entire periphery of the animal except from the posterior end. These pseudopods are of clear protoplasm except for a small number of very pale bluish granules which are frequently found at their bases. The pseudopods are broad or narrow and always blunt. They do not usually determine the direction of locomotion.

The posterior end terminates in a uroid or group of root-like pseudopodial projections attaching the organism to the substrate (fig. 6). They play a part in locomotion but just what their function is has not been carefully determined. It is certain, however, that the uroid is not necessary to locomotion to the same extent as in *P. schiedti*.

Locomotion in this form is so extremely slow that it is difficult to tell just how it is accomplished. Movement occurs at the rate of from one to two microns a minute, so that it takes from 30 minutes to an hour and a half to move the distance of its own length, or three and one-half months to creep around a baseball. There is a slight but continual and irregular movement of the endoplasm of an oscillatory sort, which, together with slight changes of body shape and the retraction and projection of pseudopods, masks the definite forward movement of the pelomyxa. Figure 9 shows that this movement undoubtedly exists. But under ordinary circumstances it is impossible to detect definite and continuous forward streaming of the endoplasm. It seems as if forward movement was the sum of all the separate local streamings.

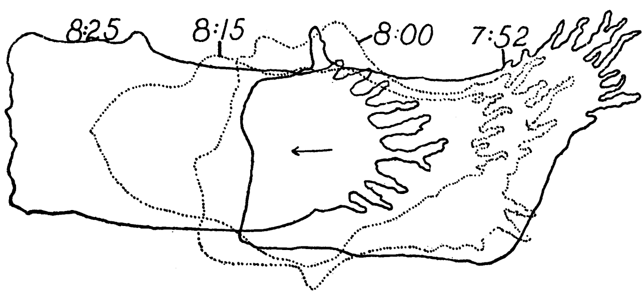

Fig. 9. Camera lucida sketch of a moving *P. lentissima* to show rate of, and form changes during, locomotion.

When suspended in the water or when loosened from the substratum this ameba is remarkable for the number of pseudopods which it throws out from all sides (fig. 7). This habit may indeed be regarded as a distinguishing characteristic of this species while in this stage. The pseudopods are usually more or less conical in shape though they always have blunt ends. The majority are of simple form, others are branched,

while some are of very odd shapes. Their length is variable, the maximum being about 50 microns. As might be expected from their rate of locomotion, the transition from a spherical shape in the suspended stage to the locomotive stage consumes much time. As the organism again begins to move, a broad wave of clear ectoplasm appears at some point near the substrate. The wave slowly enlarges as the pseudopods are withdrawn, and gradually the locomotive form reappears.

The nucleus of this species presents several points of interest. In the first place there are usually two. In perhaps three out of four individuals examined two nuclei were found. In no case did I see more than two. It seems that in this species it is the normal condition for a considerable interval of time to supervene between division of the nucleus and the division of the organism. The two processes do not seem to be directly dependent on each other. In the case of most other amebas cell division must closely follow nuclear division, or else in the majority of cases it will become impossible for cell division to take place and the animal in consequence dies (Schaeffer, '16). So that what is normal in division sequences for *Pelomyxa lentissima* is pathologic for *Amoeba proteus*, for example. A *P. lentissima* with two nuclei may be looked upon as an individual whose cytoplasm has not yet divided.

Another point of interest with regard to the nucleus of this species is that a central body is clearly observable in the living condition. The composition of this body has not been investigated. The diameter of this body is from two to two and one-half microns. Its appearance is similar to that of the chromatin granules.

The general appearance of the nucleus is somewhat like that of *P. belevskii*. It is spherical, about fourteen microns in diameter, and contains a spherical layer of chromatin granules of about eleven microns diameter. Not very much can be said about the physical character of the nuclear membrane owing to the very slight movement of the endoplasm. The nuclei are usually found somewhere near the centre of the animal.

The contractile vacuoles are numerous. In one individual there were at least sixty, but whether all were contractile or not could not be determined. The average maximum size of these contractile vacuoles is about ten microns, though many contract when only five microns or so in diameter. Occasionally one may reach fifteen microns before contraction. Consonant with the slow rate of locomotion is that of enlarge-

ment of the vacuoles. A vacuole of five or eight microns diameter may take fifteen minutes or longer to enlarge to ten microns, followed by contraction. The systole is, however, relatively rapid, occurring usually in about one second.

The spectroscope shows some very irregular small crystalline masses in the endoplasm sometimes numerous, usually comparatively few. Of "refractive bodies" (starch grains) there are only a few small ones present. Other inclusions in the ectoplasm are the bacterial rods, distinctive of the genus, reaching a length usually of four microns, very rarely of eleven microns; and the very numerous brownish masses of all sizes and shapes so common in several species of pelomyxas. In addition to these bodies there are also numerous very small greenish blue granules found in the endoplasm.

Besides an occasional diatom or shelled rhizopod, I have found no recognizable food bodies in this species.

PELOMYXA SCHIEDTI NOV. SPEC.

Diagnosis. Length in locomotion about 75 microns. Usual shape ovoidal. Color, brownish olive green, almost opaque. Pseudopods very rarely formed. Protoplasm fluid. Movement by eruptive waves of endoplasm partly reflected back along the sides. Rate of movement about 95 microns per minute. Nucleus sometimes single, usually double; spherical, about 7 microns in diameter. Chromatin granules in the form of a hollow sphere immediately under the nuclear membrane or at a slight distance from it. Contractile vacuoles small, numerous; maximum size about 4 microns; diastole rapid; systole instantaneous. Starch grains very numerous, irregular in shape, olive green in color, maximum size about 6 microns. Numerous bacterial rods present of 3 to 4 microns length. Small uroid always present during locomotion.

This species, the smallest of the pelomyxas has been found on several occasions in large numbers in old cultures of material brought from the marshes of Lonsdale (Pl. VIII, fig. 8). Because of its dark color and rapid rate of locomotion it at once attracts attention. Although three or four of my cultures were very rich in this form, it must, nevertheless, be classed as a rare species. The environment must be of a very special kind apparently in order that it may develop in numbers. It remained in my richest culture for about four weeks from the time it was first discovered. I propose for this pelomyxa the specific name *schiedti* in honor of my friend Professor R. C. Schiedt.

Under low magnification this organism appears quite black excepting at a few small places along the sides where the color is temporarily grayish owing to the accidental presence of but few of the starch grains so

abundant in this ameba. The posterior end also usually is light gray. Under high powers however, the color is of a dull brownish olive green, due to the brownish endoplasmic inclusions and the numerous starch grains. The protoplasm is bluish green.

This species passes through very slight transformations in shape during locomotion or at other times (fig. 10). Its general shape during locomotion is ovoidal with the anterior end broad, while the posterior end is narrow. Never at any time does the organism flatten itself out to any extent on the substratum. The details of the process of locomotion have not been observed carefully, but the following details may be mentioned. Owing partly perhaps to the fluid nature of the protoplasm, no pseudopods are formed for the purpose of locomotion, but broad eruptive waves of endoplasm break out somewhere near the anterior end into which then flows a part of the animal's endoplasm. These waves are usually partly reflected back along the sides of the animal, leaving a more or less clear space at the farthest point reached by the reflected wave. Occasionally for a short period the pelomyxa may also advance by endoplasmic streaming as is commonly observed in amebas generally, but the larger part of its path is negotiated by eruptive waves as described.

Another important factor in locomotion is the uroid. The animal is not attached to the substratum anywhere except at the uroid. This is readily observed when they are taken up with a capillary pipette. The anterior part of the body is readily displaced by slight currents in the water but the posterior end is not affected in this way. It seems that the thin pseudopodium like projections of which the uroid is formed are for the purpose of holding the organism in place and at the same time allow it to move forward. But just how this is done could not readily be determined owing to the fact that these uroidal projections are very small and very transparent. It has been possible to determine however, that the projections may be thrown out very rapidly, almost instantaneously, so that it is possible that new projections are continually being formed as old ones are being retracted. The alternative view is that the uroidal projections are dragged along over the substratum attaching themselves temporarily and locally as they pass over the substratum. But whatever maybe the exact rôle the uroid plays in locomotion, it is evident that the organism is prevented from rolling over by reason of its attachment to the substratum.

Although there is, as has been stated, considerable uncertainty in the direction which the waves of endoplasm take at the anterior end, the path of the organism may nevertheless, because of the activity of its prehensile uroid, be straight for a considerable distance. In a sense therefore the guiding agency in locomotion is located at the posterior end of the ameba.

The rate of locomotion in *schiedti* is rapid, being about 95 microns per minute (fig. 10).

The nucleus of this species presents nothing unusual. Its shape is spherical. The chromatin occurs in rather large masses arranged in the shape of a hollow sphere immediately underneath the nuclear membrane or at some distance from it. Usually two nuclei are present as in the case of *lentissima.* The binucleate condition therefore represents an intermediate stage between nuclear division and cell division. This stage in these two species (*schiedti* and *lentissima*) is much longer than in most typically uninucleate unicellulars, so that the binucleate stage is much more common. These pelomyxas are therefore to be looked upon not as binucleate organisms, but as typically uninucleate. The size of the nucleus is about 7 microns. Owing to the numerous endoplasmic inclusions, the nuclei are difficult to see in the living condition.

The contractile vacuoles are made out only with the greatest difficulty in normal individuals. They are seen only in the small clear areas which are observed occasionally along the sides during locomotion. With very attentive examination the vacuoles may then be seen. The maximum size of the vacuoles is about four microns. Nothing very definite can be said about their number which is certainly not less than ten, but is probably very much greater. The diastole is rather rapid. The systole is practically instantaneous, almost as rapid as the bursting of a bubble on the surface of water. There is a readily observed characteristic rush of protoplasm from the immediate vicinity to the place where the vacuole has just burst, which may be taken advantage of to locate a bursting vacuole without actually seeing the vacuole. In this way it is possible to determine that there are several systoles a minute in different parts of the animal. Doubtless the fluidity of the endoplasm is in some way connected with the small size and sudden contraction of the vacuoles.

This species is full of what are probably glycogen grains (Stolc, A., 1900. Zeit. f. wiss. Zool. Bd. 68). Their color is a shade of olive green.

The shapes of the bodies are varied, mostly irregular, angular with rounded corners and edges. The maximum size commonly met with is about six microns. Most of them are only two or three microns long. They are not evenly distributed throughout the body, but there seems to be a tendency for them to collect very near the surface in what is called the ectoplasm. In focussing along the edge one observes a serrated outline, the teeth being represented by the protruding starch grains. I presume that a layer of protoplasm at all times covers these bodies when lying at the surface, though one cannot observe such a layer in the living organism.

Besides the starch bodies there are found considerable numbers of very small spherical bodies of a bluish green color. These are met with in nearly all amebas, but of their nature nothing is definitely known.

The bacterial rods, the presence of which characterizes the genus *Pelomyxa*, are found in considerable numbers in *schiedti*. The length of these rods is about three or four microns.

The number of brownish colored inclusions which are so commonly found in pelomyxas, is small in this species. Sometimes only two or three masses of appreciable size are found. Very little food has been observed in the bodies of these animals. Occasionally a diatom or a flagellate was seen, but in the great majority of individuals no recognizable food objects were found.

When the cultures began to die out, the glycogen bodies began to disappear gradually. In the last few surviving individuals almost no glycogen grains could be seen. The organisms were very pale yellowish and sluggish. Numerous large permanent vacuoles appeared. The nuclei also changed in appearance. The chromatin receded further from the nuclear membrane and collected itself in much larger but fewer granules than normally. From these observations we may conclude that the starch grains are reserve food stores as has been shown by Stolc to be the case in *P. palustris*, and that the cultures died out chiefly because of lack of food.

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EXPLANATION OF PLATES

Plate VII

Fig. 2. Photograph from water color drawing of *bigemma* in characteristic attitude during locomotion. C, nuclear chromatin mass; C-S, crystals attached to spheres; F, food mass; M, nuclear membrane; V vacuoles.

Fig. 3. Photograph from water color drawing of nucleus and excretion spheres and crystals of *bigemma*. CR, chromatin granules of nucleus; C, twin crystals at-attached to excretion spheres; M, nuclear membrane; S, spheres; Occasionally two twin crystals are attached to each other as shown.

Fig. 4. Photomicrograph from unretouched negative of several live *bigemmas* among diatoms and arcellas. The nucleus, N, is faintly shown in two of them. The arcellas measured 58 microns in diameter.

Fig. 5. Photomicrograph from unretouched negative of several live *bigemmas* in characteristic attitudes during locomotion. Note especially the blunt character of the ends of the pseudopods. Same magnification as figure 4.

Plate VIII

Fig. 6. Photograph from water color sketch of a *P. lentissima* in locomotion. N, nucleus; CV, contractile vacuole; U, uroid.

Fig. 7. Water color sketch of a quiescent stage of *lentissima*. Note the numerous and bizarre shaped pseudopods.

Fig. 8. Water color sketch of *P. schiedti* in locomotion. CV, contractile, vacuole; N, nuclei; U, uroid. Note the numerous irregularly shaped starch bodies.

Fig. 10. Camera lucida sketch of a moving *P. schiedti* showing the slight changes in body shape during locomotion.

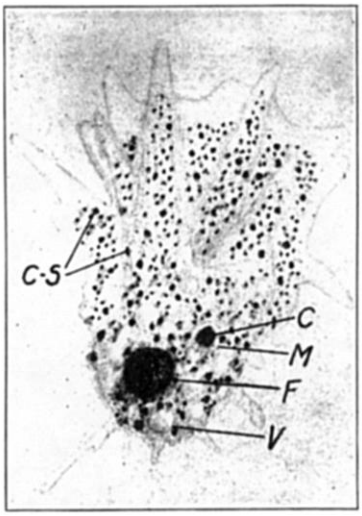


FIG. 2

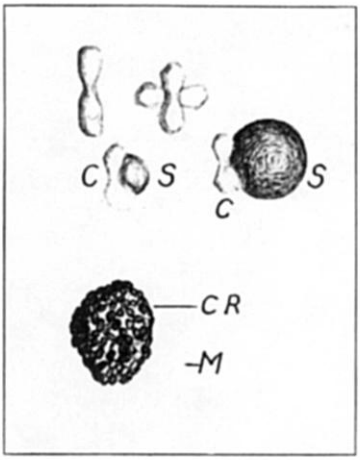


FIG. 3

FIG. 4

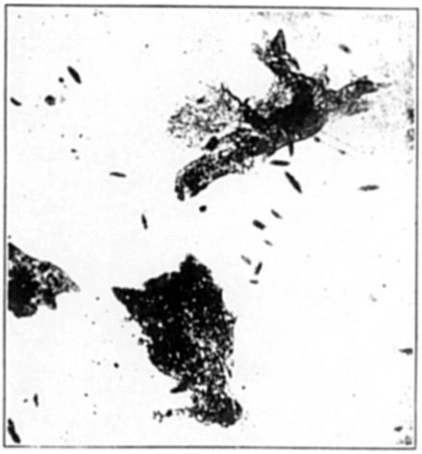


FIG. 5

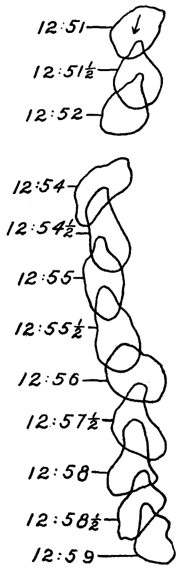
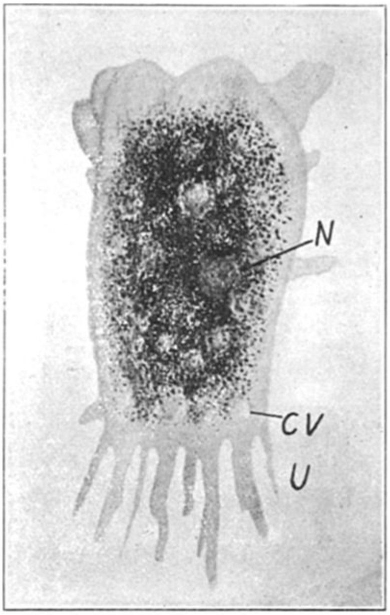


FIG. 10



FIG, 6

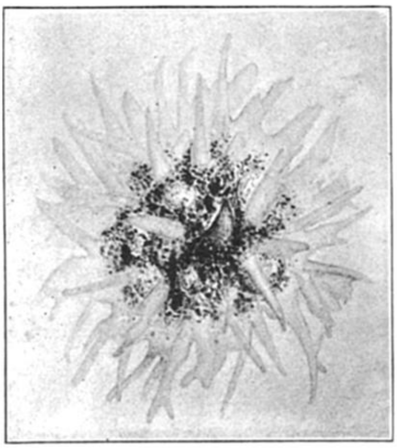


FIG. 7

FIG. 8